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Growth and production of Thai agarophyte cultured in natural pond using the effluent seawater from shrimp culture

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Key words: growth, natural culture pond, production, Thai agarophyte

Abstract

Growth rate determinations of the Thai agarophytes, *Gracilaria fisheri* (Xia *et* Abbott) Abbott, Zhang *et* Xia and *G. tenuistipitata* Chang *et* Xia var. *liui* Chang *et* Xia, were conducted by monoline method in natural earthen ponds (800 m^2 in area) using shrimp pond effluents (P1) and ambient seawater (P2), from January 1998 to July 1999. Generally, plants of both species cultured in P1 showed a better growth rate and total production than those cultured in P2. Growth rates and total production of the *Gracilaria* cultured in P1 increased in the rainy months and reached a maximum value of $3.08 \pm 1.14\%\ d^{-1}$ for *G. fisheri* and $2.68 \pm 1.76\%\ d^{-1}$ for *G. tenuistipitata* in January 1999. In contrast, growth of both species cultured in P2, projected a slight change in their growth rates, with a maximum value of $1.85 \pm 1.00\%\ d^{-1}$ for *G. fisheri* and $1.70 \pm 0.49\%\ d^{-1}$ for *G. tenuistipitata* attained in the rainy period (August 1998). All plants of *G. tenuistipitata* declined drastically in the following dry season. Total production of *G. fisheri* and *G. tenuistipitata* cultured in P1 showed the highest value of 1000 g wet wt and 961 g wet wt in January 1999, respectively. Plants of both species showed fluctuation in growth and total production, depending on specific cultured conditions of each pond, algal strain used, and on the season. The results suggest that *G. fisheri* can be grown all year round and is more suitable than *G. tenuistipitata* for earthen pond cultivation using shrimp pond effluents.

Introduction

Thailand is an entirely tropical monsoon country, pronounced wet and dry seasons characterize the climate. The rainy season of the south-west monsoon is usually established from May to September, whereas the dry season of the north-east monsoon begins in November and ends in February (Lewmanomont, 1998). Characteristics of the coast of Thailand are mainly mangrove swamps and muddy to sandy beaches.

In the past few years, intensive cultivation of shrimp has expanded considerably in many coastal areas of Thailand. The activities of shrimp cultivation continue almost throughout the year, except for the cold months (December and January) when there is a decrease in shrimp growth. The increase of the intensive farm activities has affected the coastal environment of the country. This is because waste waters from the sea farms usually contain large amounts of nitrogen excreted by the shrimps. Several investigations have considered the nutrient-rich effluents as a nitrogen source in mariculture (Vandermeulen & Gordin, 1990; Haglund & Pedersén, 1993; Jiménez del Rio et al., 1996; Yamasaki et al., 1997). They suggested that available nutrient-rich effluents could enhance the growth of marine algae.

Integrated cultivation of the Thai agarophyte *Gracilaria fisheri* (Xia *et* Abbott) Abbott, Zhang *et* Xia with fishes has been attempted in outdoor tanks and ponds (Singthaweesak, 1995, 1996). However, production of the agarophyte has not been successful. There have been very few reports on seaweed growth in Thailand, particularly on its seasonal changes in relation to environmental factors.

The present study was carried out to ascertain variations in growth of the Thai agarophytes, *Gracilaria fisheri* and *G. tenuistipitata* Chang *et* Xia var. *liui* Chang *et* Xia cultured under natural earthen pond

Table 1. Chemical and physical characteristics of the seawater used for cultivation of *Gracilaria fisheri* and *G. tenuistipitata* in natural earthen ponds from January 1998 to July 1999. P1= shrimp pond effluents, P2 = ambient seawater

Date		Temperature (°C)		Salinity ‰)		Turbidity (mg l^{-1})		Alkalinity (mg l^{-1})		PO_4-P (mg l^{-1})		DIN (mg l^{-1})	
		P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
1998	Jan.	30.7	31.0	31.0	35.0	–	–	81	110	0.01	0.01	0.24	0.22
	Jan.	31.0	31.0	31.0	34.0	–	–	77	127	0.01	0.01	0.21	0.21
	Feb.	30.0	31.0	30.0	33.0	–	–	116	170	0.01	0.02	0.24	0.16
	Feb.	31.0	29.0	31.0	32.0	–	–	131	118	0.00	0.01	0.22	0.20
	Feb.	32.0	32.0	35.0	40.0	–	–	160	173	0.05	0.03	0.50	0.20
	Feb.	29.0	29.0	38.0	40.0	–	–	167	178	0.05	0.02	0.33	0.14
	Mar.	31.0	31.0	28.0	32.0	–	–	296	243	0.03	0.01	0.18	0.19
	Mar.	31.0	29.0	32.0	32.0	–	–	241	232	0.04	0.04	0.18	0.16
	Mar.	33.0	33.0	34.0	38.0	–	–	198	217	0.03	0.02	0.18	0.13
	Apr.	35.0	34.0	26.0	27.0	–	–	244	255	0.02	0.02	0.29	0.20
	May	34.5	34.0	34.0	30.0	–	–	147	234	0.04	0.02	0.33	0.22
	May	34.0	35.0	28.0	26.0	–	–	146	227	0.03	0.02	0.36	0.34
	Jun.	33.0	32.5	30.0	27.0	–	–	148	204	0.04	0.04	0.41	0.26
	Jun.	33.0	32.5	26.0	30.0	–	–	155	153	0.06	0.05	0.44	0.19
	Jun.	33.0	32.5	20.0	34.0	–	–	182	119	0.03	0.05	1.45	0.29
	Jul.	32.0	33.0	22.0	30.0	–	–	146	110	0.06	0.04	0.37	0.26
	Jul.	31.0	30.0	35.0	31.0	–	–	158	144	0.06	0.05	0.78	0.23
	Jul.	34.0	35.0	25.0	30.0	50.57	14.54	150	109	0.06	0.05	0.50	0.20
	Jul.	32.0	31.0	29.0	35.0	44.91	14.77	117	103	0.04	0.07	2.20	2.53
	Aug.	30.0	30.0	25.0	30.0	49.87	13.31	125	132	0.05	0.05	0.59	0.50
	Aug.	30.0	31.0	25.0	29.0	50.39	24.53	160	135	0.05	0.04	0.18	0.15
	Aug.	21.0	21.0	20.0	21.0	109.31	31.12	89	100	0.02	0.02	0.28	1.25
	Aug.	25.0	25.0	23.0	25.0	59.56	47.53	120	125	0.02	0.02	0.29	0.20
	Sep.	19.0	19.0	28.0	22.0	54.54	43.39	131	125	0.06	0.04	1.30	0.26
	Sep.	19.0	18.0	25.0	27.0	38.89	23.53	97	91	0.02	0.02	0.22	0.21
	Sep.	23.0	22.0	25.0	25.0	38.07	14.77	143	126	0.02	0.03	0.27	0.22
	Sep.	31.0	31.0	25.0	28.0	40.47	28.32	143	150	0.02	0.03	0.16	0.17
	Sep.	31.0	32.0	25.0	27.0	37.66	23.36	109	155	0.02	0.03	0.29	0.11
	Sep.	32.0	32.0	24.0	23.0	52.61	44.91	124	143	0.02	0.02	0.32	0.18
	Oct.	32.0	32.0	26.0	20.0	29.26	39.83	113	110	0.04	0.05	0.20	0.17
	Oct.	29.0	31.0	20.0	23.0	27.15	16.29	94	150	0.01	0.01	0.14	0.26
	Oct.	29.0	30.0	20.0	26.0	43.50	36.26	63	80	0.01	0.01	0.15	0.16
	Nov.	29.0	29.0	20.0	30.0	25.81	37.20	99	141	0.01	0.01	0.23	0.11
	Nov.	26.0	26.0	25.0	30.0	14.60	36.15	86	93	0.01	0.01	0.15	0.12
	Dec.	32.0	32.0	25.0	30.0	21.55	40.29	53	124	0.01	0.00	0.15	0.14
	Dec.	30.0	30.0	25.0	30.0	33.29	39.01	85	71	0.00	0.01	0.12	0.15
	Dec.	27.0	27.0	27.0	30.0	32.33	39.94	117	110	0.00	0.00	0.11	0.50
	Dec.	24.0	24.0	26.0	33.0	40.12	41.64	82	85	0.00	0.00	0.11	0.14
1999	Jan.	34.0	34.0	30.0	35.0	40.41	56.00	153	166	0.01	0.01	0.14	0.12
	Jan.	33.0	33.0	32.0	35.0	41.11	69.84	88	128	0.00	0.00	2.55	0.31
	Feb.	30.0	30.0	34.0	37.0	73.99	81.23	92	105	0.00	0.00	0.42	0.46
	Feb.	31.0	31.0	33.0	37.0	80.94	72.82	85	60	0.00	0.40	0.39	0.40
	Mar.	30.0	30.0	35.0	40.0	110.36	23.18	71	52	0.00	0.00	0.41	0.81
	Mar.	28.0	27.0	40.0	44.0	129.17	43.74	121	100	0.00	0.00	0.40	0.37
	Apr.	32.0	32.0	35.0	40.0	162.22	68.79	165	108	0.00	0.00	0.07	0.09
	Apr.	30.0	30.0	40.0	45.0	29.08	47.18	160	121	0.01	0.00	0.05	0.04

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Table 1. Continued

Date	Temperature	(°C)	Salinity	‰)	Turbidity	($mg\ l^{-1}$)	Alkalinity	($mg\ l^{-1}$)	PO_4-P	($mg\ l^{-1}$)	DIN	($mg\ l^{-1}$)
	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
May	29.0	29.0	35.0	35.0	34.16	26.48	160	126	0.00	0.00	0.04	0.27
Jun.	30.0	30.0	38.0	40.0	46.48	61.14	77	105	0.00	0.00	0.02	0.02
Jun.	29.0	29.0	40.0	37.0	45.02	60.96	100	83	0.00	0.00	0.0 2	0.02
Jun.	35.0	35.0	40.0	38.0	25.58	69.66	139	112	0.01	0.00	0.11	0.12
Jun.	31.0	32.0	37.0	42.0	38.89	72.53	146	116	0.01	0.01	0.12	0.14
Jul.	33.5	34.0	35.0	38.0	34.69	70.60	133	106	0.01	0.00	0.11	0.09
Jul.	30.0	30.0	35.0	38.0	29.02	47.30	135	120	0.01	0.01	0.23	0.08
Jul.	32.0	31.0	32.0	33.0	32.29	38.42	135	118	0.01	0.01	0.14	0.06
Jul.	32.0	32.0	36.0	40.0	35.68	34.28	130	123	0.01	0.01	0.08	0.06

conditions, using shrimp pond effluents and ambient seawater. Annual growth and production of the algae with changes in environmental conditions, and their chemical compositions (chlorophyll, r-phycoerythrin, C:N ratio) were determined.

Materials and methods

Natural earthen pond conditions and plant material

Seaweed cultivation was conducted in natural earthen ponds at the Phetchaburi Coastal Aquaculture Station, Phetchaburi province (100° 05′ 15″ E, 13° 02′ 30″ N), 250 km south of Bangkok, Thailand. Two ponds (P1 and P2), originally constructed for cultivation of fish, were used in the experiments. The ponds were 800 m^2 in area and 150 cm deep. The seawater supply was from an extensive shrimp culture pond and from ambient seawater. In Experiment 1 the shrimp pond effluents of 350 m^3 were filtered with a net (2 mm mesh size) and mixed with 650 m^3 of ambient seawater in P1. In Experiment 2 the ambient seawater of 1000 m^3 was pumped directly through the filter net into P2. Aeration was provided with high-flow compressors supplying air to the different ponds, each having six PVC pipes (10 m length) with outlet, and placed in an interval of 1 m on the bottom. The water exchanges of 70% volume were conducted every 7–14 days.

Gracilaria fisheri and *G. tenuistipitata* var. *liui* were collected from Pattani Bay, in the south-east coast of the Gulf of Thailand. Plants were pre-cultivated by monoline method (Trono, 1987) for 6 months under both pond conditions mentioned above, prior to the start of the experiments. Fifty algal samples (5–10 g $sample^{-1}$) were inserted between the braids of the nylon rope (10 m length) at an interval of 15 cm $sample^{-1}$. Pre-culture and culture experiments were performed in the ponds with initial stocking weight of 500 and 250 g wet wt $rope^{-1}$ or 5.0 and 2.5 kg wet wt $pond^{-1}$, respectively, at 100 cm water depth. Algae were suspended in the water column at 15 cm above the muddy bottom.

Harvesting, growth rate and production

Cultivars were harvested every 7–14 days and re-stocked to the same initial weight. Relative daily growth rates were calculated according to the equation (Penniman et al., 1986):

$$RGR = \{(W_t/W_0)^{1/t} - 1\} \times 100,$$

where RGR = relative daily growth rate; W_0 = initial fresh weight; W_t = final fresh weight; and t = time (days). Total production of the algae in g fresh wt was monitored monthly.

Laboratory analyses

During the experiments, determination of surface seawater temperature, salinity, turbidity, alkalinity, PO_4-P and total dissolved inorganic nitrogen (ammonia + nitrite + nitrate) were made as one point measurements every working day. Water temperature and salinity were measured using an alcohol thermometer, and refracto-salinometer, respectively. Turbidity of the seawater was recorded at 420 nm with a spectrophotometer (Shimadzu UV-1601). Alkalinity and ammonia-N were determined according to APHA, AWWA &

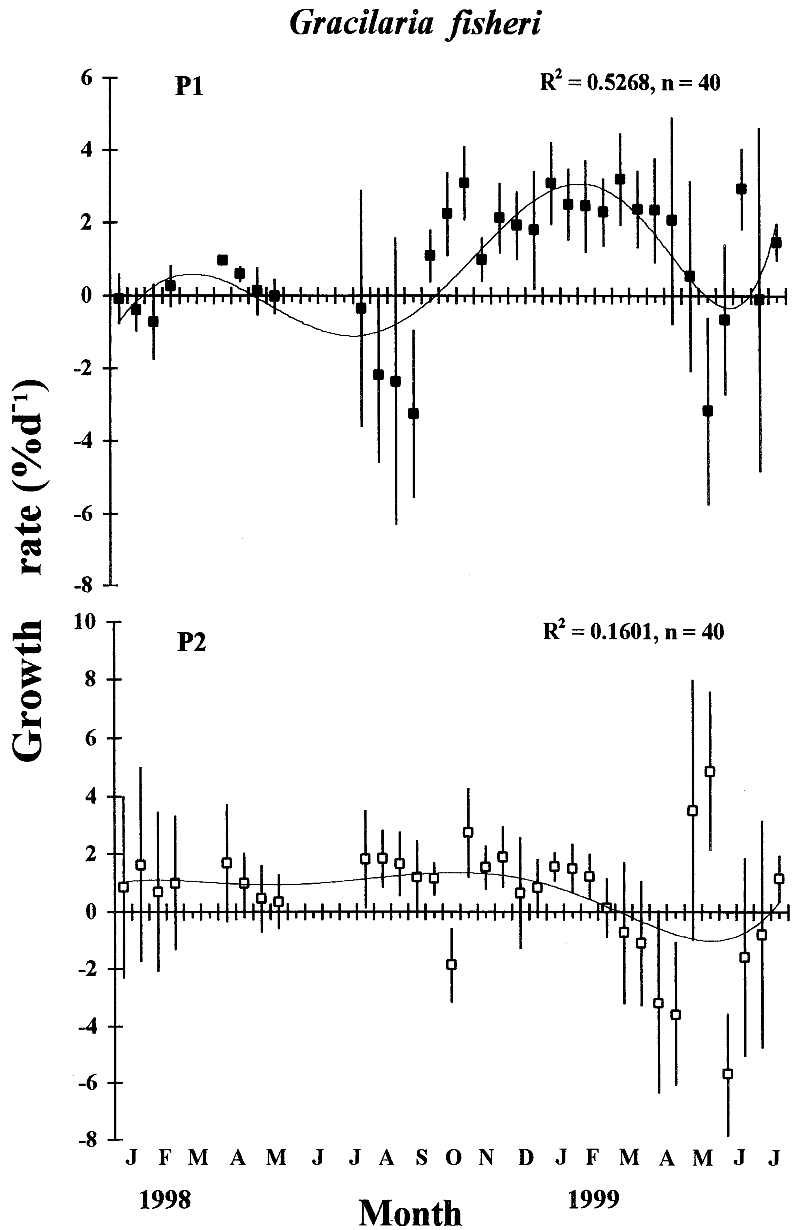

Figure 1. Mean growth rate (± SD) of *Gracilaria fisheri* cultured in natural earthen pond conditions using shrimp pond effluents (P1) and ambient seawater (P2) from January 1998 to July 1999. The growth curve (solid line) is best fitted using the polynomial regression ($n = 40$).

WPCF (1980) and PO_4-P, NO_3-N and NO_2-N according to Strickland & Parsons (1972). Determinations of nitrogen and organic carbon concentration, and C:N ratio in the algal tissue were performed with method described by Walsh & Beaton (1973). Samples of fresh material were analyzed for total chlorophyll (Mackinney, 1941) and for r-phycoerythrin (MacColl & Guard-Friar, 1987).

Results

The chemical and physical characteristics of the surface seawater in both experiments are shown in Table 1. Annual temperature of surface seawater in both P1 and P2 ranged from 18 °C in the late rainy season (September) to 35 °C in the dry season (April). Salinity generally varied from 20‰ to 35‰ in both P1 and P2, but increased drastically (36–45‰) during dry periods. Turbidity varied from 14.60 to 162.22 mg l^{-1}

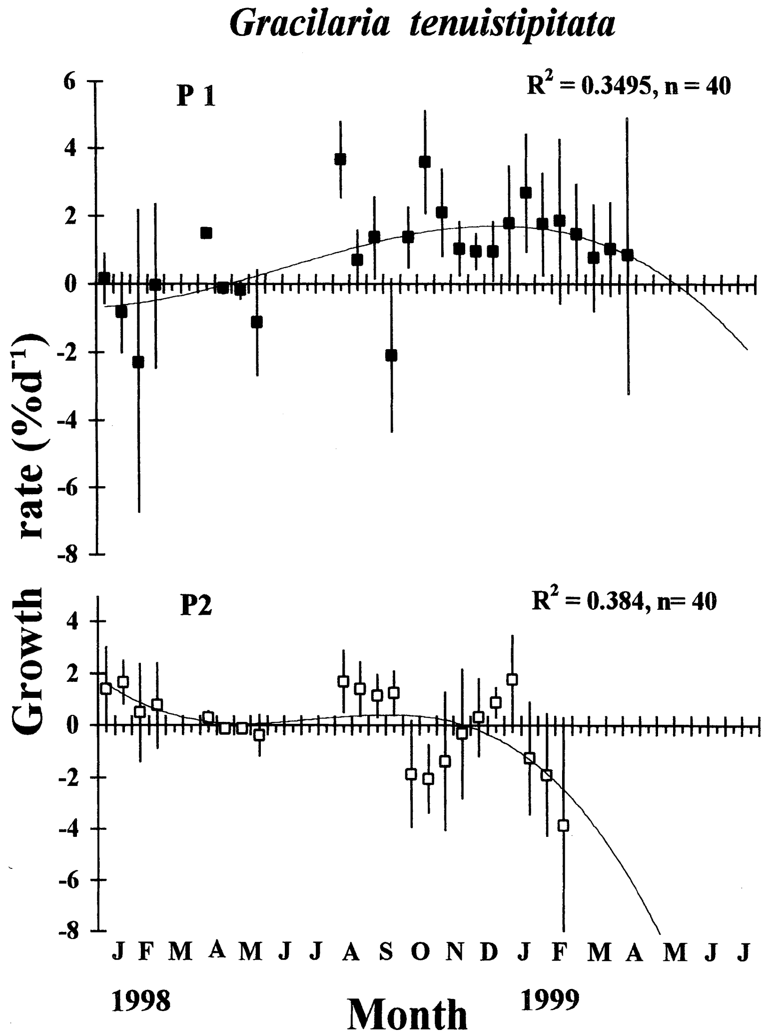

Figure 2. Mean growth rate (± SD) of *Gracilaria tenuistipitata* cultured in natural earthen pond conditions using shrimp pond effluents (P1) and ambient seawater (P2) from January 1998 to July 1999. The growth curve (solid line) is best fitted using the polynomial regression (n = 40).

in P1 and 13.31 to 81.23 mg l^{-1} in P2. In the natural earthen pond, total dissolved inorganic nitrogen (DIN) concentration was generally higher in P1 than in P2 with values ranging from 0.02 to 2.55 mg l^{-1} in P1 and 0.02 to 2.53 mg l^{-1} in P2. PO_4-P concentration was from <0.01 to 0.06 mg l^{-1} in P1 and <0.01 to 0.40 mg l^{-1} in P2. Alkalinity ranged from 53 to 296 mg l^{-1} in P1 and 52 to 255 mg l^{-1} in P2.

Cultivars of *Gracilaria fisheri* and *G. tenuistipitata* showed fluctuation in their growth rates throughout the year (Figs 1 and 2). Both agarophytes exhibited variations in growth depending on season, algal strain and specific culture conditions of each pond. Growth of the agarophytes was rather low at the beginning of culture period (January–April 1998), and continued to worsen during unusual dry period due to El Nino, increasing water salinity (>45‰) and temperature (>30 °C). Cultivars grew again when water salinity and temperature decreased during rainy (August 1998) and cold months (November 1998–January 1999).

Higher growth rate of *Gracilaria* cultured in P1, as indicated by the fitted polynomial regression line, was obtained in the rainy period (August–October 1998) when salinity was between 20–28‰ in P1 and 20–30‰ in P2. A maximum value for *G. fisheri* (3.08 ± 1.14% d^{-1}; r^2 = 0.53; n = 40) and for *G. tenuistipitata* (2.68 ± 1.76% d^{-1}; r^2 = 0.35; n = 40) was reached in January 1999. Conversely, the regression growth curve of the *Gracilaria* cultured in P2 indicated a peak of maximum growth rate for *G. fisheri* (1.85 ± 1.00% d^{-1}; r^2 = 0.16; n = 40) and for *G. tenuistipitata* (1.70 ± 0.49% d^{-1}; r^2 = 0.38; n = 40)

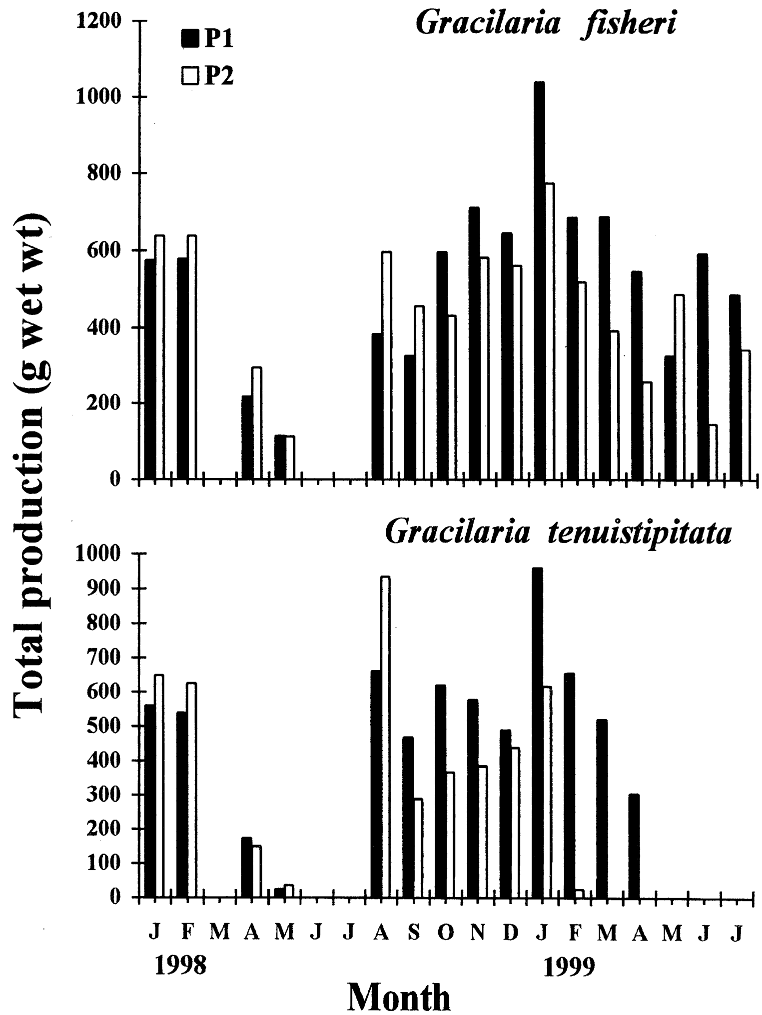

Figure 3. Changes in the total production of *Gracilaria fisheri* and *G. tenuistipitata* cultured in natural earthen pond conditions using shrimp pond effluents (P1) and ambient seawater (P2) from January 1998 to July 1999.

in the rainy season (August 1998). Growth of both species decreased gradually, then drastically when water salinity exceeded 35‰, temperature over 30 °C and alkalinity >160 mg l^{-1}. *G. fisheri* cultivars could grow and acclimatize to the new culture conditions, but not for *G. tenuistipitata*.

Total production (Fig. 3) of cultivars in P1 obtained higher value than those in P2. *Gracilaria fisheri* showed the highest production of 1000 g wet wt (P1) and 777 g wet wt (P2) in January 1999 while those of *G. tenuistipitata* were 936 g wet wt in August 1998 (P2) and 961 g wet wt in January 1999 (P1). However, production of the algal wet weight was higher in P2 than in P1 during the early period of the study (January–September 1998).

Total chlorophyll content (Fig. 4) was higher for the cultivars in P1 than those in P2. The value for *Gracilaria fisheri* ranged from 0.25 to 1.19 mg g^{-1} tissue in P1, 0.27 to 0.97 mg g^{-1} tissue in P2, and for *G. tenuistipitata*, 0.35 to 1.37 mg g^{-1} tissue in P1 and 0.28 to 1.29 mg g^{-1} tissue in P2. The maximum values were obtained in the summer month of March 1999. The content of r-phycoerythrin (Fig. 5) in *G. tenuistipitata* increased during rainy and early cold months, and gradually decreased further towards summer. The polynomial regression curve of *G. fisheri* and *G. tenuistipitata* indicated the content of r-phycoerythrin to correspond with the growing period of the algae.

Changes in the tissue C:N ratios of *Gracilaria fisheri* and *G. tenuistipitata* are illustrated in Figure 6. The C:N ratio in *G. fisheri* ranged from 7.51 to 40.26 in P1 and 9.54 to 51.74 in P2, and in *G. tenuistipitata* from 5.51 to 14.90 in P1 and 6.35 to 19.03 in P2. These

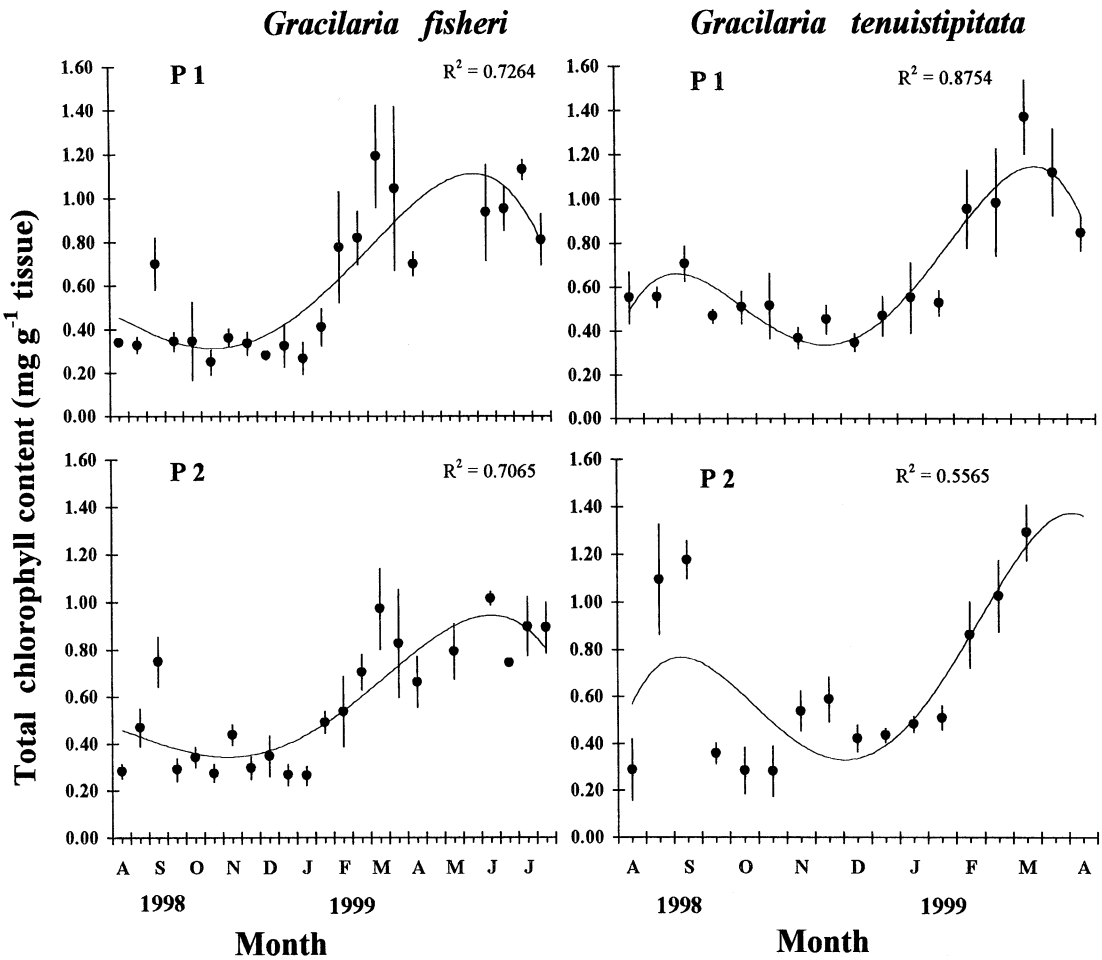

Figure 4. Mean total chlorophyll content (± SD) of *Gracilaria fisheri* and *G. tenuistipitata* cultured in natural earthen pond conditions using shrimp pond effluents (P1) and ambient seawater (P2), from August 1998 to July 1999. The overall pattern of changes in total chlorophyll content (solid line) is best fitted using the polynomial regression ($n = 5$).

ratios in both species were generally lower in P1 than in P2, which corresponded to the higher growth of the plant cultured in P1. However, relationship between the tissue C:N ratios, nutrient and growth rate was not clear among the *Gracilaria* species.

The main interfering epiphytes in our experiments were the blue green alga, *Lyngbya majuscula*, the green algae, *Enteromorpha clathrata*, *Chaetomorpha crassa*, and *Cladophora prolifera*, the seagrass, *Ruppia maritima* and other animals (e.g. tube worm, copepods). They proliferated on the *Gracilaria* and on the bottom of the culture ponds. The epiphytes entangled, entrapped and caused the *Gracilaria* to float, making the plants vulnerable to the high surface summer temperatures. The other animals also entrapped and damaged to tissue of the *Gracilaria*.

Discussion

Growth rate and chemical compositions of *Gracilaria* spp. in outdoor cultures have been reported as a function of salinity, temperature, irradiance, nitrogen and phosphate concentration, plant density, rate of water exchange, aeration, pH and inorganic carbon supply (Lapointe & Ryther, 1979; Lapointe, 1981, 1987; Friedlander et al., 1991; Friedlander et al., 1993; Gonen et al., 1993). Positive correlations between growth rates and nitrogen content have been shown in outdoor cultivation tanks of other *Gracilaria* species (Haglund & Pedersén, 1993; Jiménez del Rio et al., 1996; Yamasaki et al., 1997). With respect to the present study, growth rates of *G. fisheri* and *G. tenuistipitata* increased in response to the effluents from extensive shrimp culture pond. The C:N ratio indicated available nutrient in the shrimp pond effluents

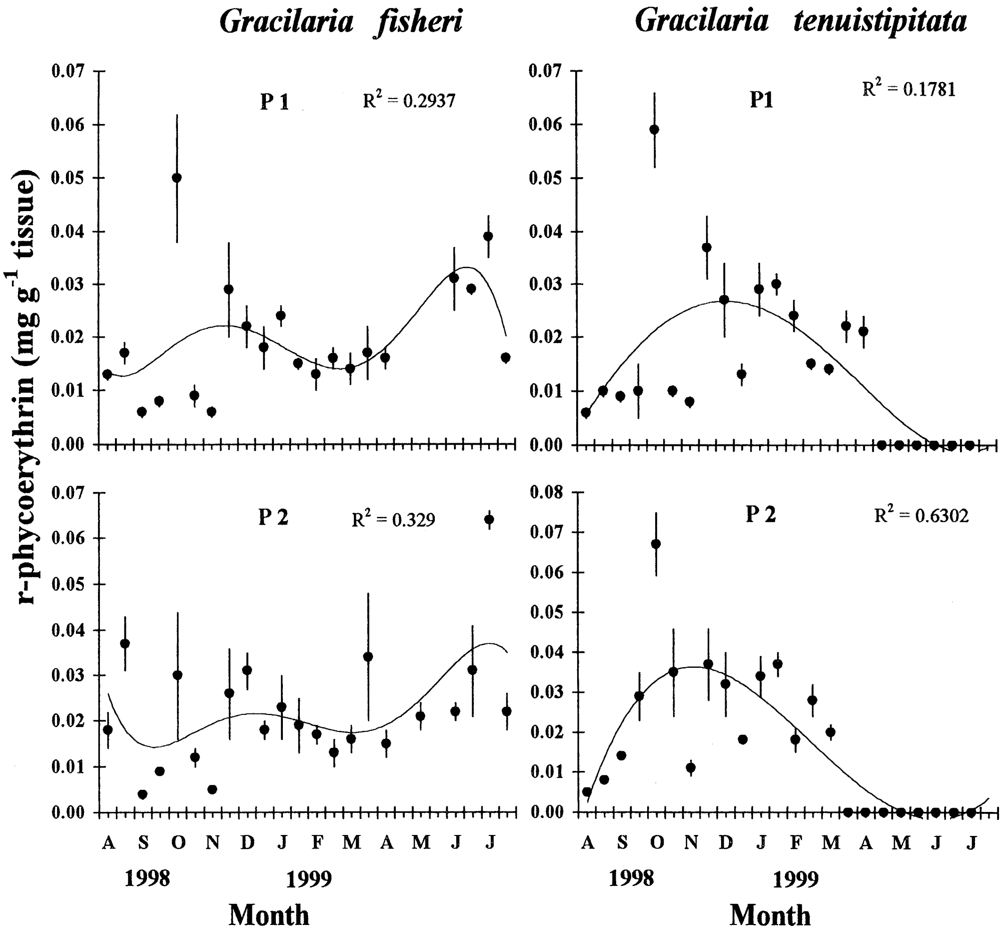

Figure 5. Mean concentration of r-phycoerythrin (± SD) in *Gracilaria fisheri* and *G. tenuistipitata* cultured in natural earthen pond conditions using shrimp pond effluents (P1) and ambient seawater (P2), from August 1998 to July 1999. The overall pattern of changes in concentration of r-phycoerythrin (solid line) is best fitted using the polynomial regression ($n = 5$).

sufficient for growing the Thai agarophytic *Gracilaria*. This result seems to have no N-limitation effect on the algal growth under the natural earthen pond conditions. Lower C:N ratios obtained in the tissues of both *Gracilaria* species in P1 than in P2, could be caused by the accumulation of nitrogen compounds in the tissues of plants which is related to higher nutrient-N in P1 (Friedlander & Levy, 1995). Tissue C:N ratio of about 6 indicated good growth in *Gracilaria secundata* grown at 25 °C under laboratory conditions in Uppsala, Sweden (Lignell & Pedersén, 1987). However, a C:N ratio of about 29 had been reported as an indicator of normal growth of *Kappaphycus alvarezii* in the open sea cultivation (Rui et al., 1990). A tissue C:N ratio in *G. tikvahiae* higher than 13.5 has been suggested as a sign of nitrogen deficiency which reduces growth (Hanisak, 1987). Friedlander et al. (1991) reported that the C:N ratio had an inverse relationship with the ammonium concentration of the culture medium and with growth rate. Under the natural earthen pond conditions, however, the tissue C:N ratio of each *Gracilaria* species was not clearly related with the growth rate. An increment in growth rate and total production of the *Gracilaria* species in P1, observed in January 1999, could be caused by increments in DIN concentration during that period. In addition, higher production of the algae in P2 than in P1 obtained during the early period of culture (January–September 1998), may be caused by lower turbidity of the seawater used (higher light intensity) in P2 than in P1 associated with interrupted aeration in P1.

Furthermore, variations in the growth and population of both *Gracilaria* species were also dependent on season and strain of the algae used in the culture. Similar results had been reported in long-term experiments in outdoor culture of *Gracilaria verrucosa* strain G-16 in Florida (Bird & Ryther, 1990), *G. conferta* in Israel (Levy et al., 1990), and *G. tenuistipitata* in Sweden

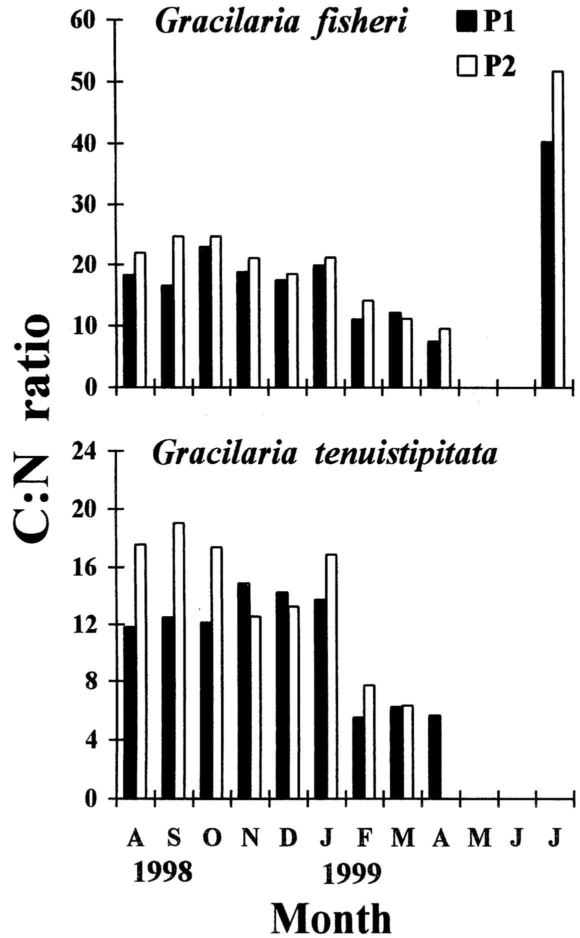

Figure 6. Tissue C:N ratio in *Gracilaria fisheri* and *G. tenuistipitata* cultured in natural earthen pond conditions using shrimp pond effluents (P1) and ambient seawater (P2), from August 1998 to July 1999.

(Haglund & Pedersén, 1993). Difference in pigment composition of the *Gracilaria* cultured in P1 and P2 likely arose in response to changes in environmental conditions (Fredriksen & Rueness, 1989). Although there may be general trends in growth due to seasonal changes in climatic factors, there also appears to be exceptions. Markedly higher growth rates were found in some months (Figs 1 and 2), probably due to the better growth of the freshly re-stocked samples after the older algae had declined in growth during the dry periods.

Weekly fluctuations in growth of *Gracilaria fisheri* and *G. tenuistipitata* may also be explained by the competitive growth of epiphytes (Hanisak, 1987; Friedlander et al., 1991). In addition, reduction in the growth rate and production of the algae may be caused by extremes in salinity, temperature and alkalinity, in dry periods. A weekly addition of seawater and low rate of water exchange in some months, during growing period, may also be insufficient to maintain growth in the summer. However, a previous report by Vandermeulen & Gordin (1990) showed that continuous waste water flow through had not provided better growth of *Ulva* in culture tank.

These present results provide further evidence that the Thai agarophytes can be grown under natural pond conditions using shrimp pond effluents all year round. *Gracilaria fisheri* showed a better growth, suggesting that this species could be more suitable than *G. tenuistipitata* for outdoor cultivation in Thailand. However *G. tenuistipitata* can be cultivated under natural pond conditions for 6–7 months of the year. Growth of the algae may potentially be improved by a higher rate of water exchange as mentioned by Freidlander & Levy (1995).

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